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Prolonged Transit Time through the Stomach and Small Intestine Improves Iron Dialyzability and Uptake in Vitro

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The iron dialyzability and uptake in relation to transit time through the stomach and small intestine was investigated using a dynamic in vitro gastrointestinal model in combination with Caco-2 cells. Three test meals were evaluated, consisting of lactic fermented vegetables with white (I) or whole meal bread (II) and of sourdough-fermented rye bread (III). Three transit times were tested (fast, medium, and slow transport). Iron dialyzability and absorption differed significantly between medium and slow transit time for meal I and between fast and medium transit time for meal III. For meal II, high in phytate, the iron dialyzability and absorption were low irrespective of transit time. The meals could be ranked with respect to iron dialyzability and uptake in the order I > III > II. Although the in vitro models used have limitations compared to in vivo experiments, the results suggest that an increased transit time may improve iron availability.

KEYWORDS: Iron dialyzability; iron absorption; transit time; gastrointestinal model; Caco-2 cells

INTRODUCTION

Iron deficiency is one of the greatest nutritional issues in the world today. According to WHO (*1*), as many as 4−5 billion people may suffer from iron deficiency, with negative consequences for physical health as well as mental development (*2*). Numerous studies have focused on counteracting this problem through various ways, which include applying different food processing methods, iron fortification, and supplementation.

The amount of iron absorbed from a meal depends on several factors, such as the iron status of the individual and the composition of the meal. There may be both enhancers (e.g., ascorbic acid and meat) and inhibitors (e.g., phytate and polyphenols) of iron absorption in a meal (reviewed in ref *3*).

There have only been a limited number of studies on the effect of gut transit time on iron absorption. The rate of gastric emptying may play a significant role in iron availability measurements, since it may take up to 1 h in acid solution to convert insoluble iron hydroxide complexes to a soluble form (*4*). Powell et al. (*5*) also proposed that the longer the time spent in the stomach and small bowel, probably the better the opportunity of dissolving and absorbing an ingested mineral. The fact that the absorption of minerals from beverages sometimes is lower than that from solid foods could be explained by the shorter transit time for liquids in the stomach, leaving less time for developing a sufficiently acidic gastric juice for the complete dissolution of some minerals (*6*). A study on iron-deficient rats showed that the time for iron uptake was prolonged as a result of slower gastric emptying (*7*).

Gastric emptying and transit time of food through the gastrointestinal tract have mainly been studied for other purposes than iron absorption. One main area of interest during the last years has been the glycemic response to a meal, which is correlated to the insulin levels in the blood. High levels of insulin are a risk factor for the development of several diseases, such as type-2 diabetes and atherosclerosis. Several researchers have observed a relation between reduced post-prandial blood glucose and insulin response after a meal and a decreased rate of gastric emptying (*8*−*11*).

The literature on the actual effect of prolonged transit time on iron uptake is scarce, probably because a study of this kind is hard to conduct on humans since the means of controlling the transit time is limited. As a very useful alternative a computer-controlled in vitro model of the stomach and small intestine has been developed at TNO Nutrition and Food Research in Zeist, The Netherlands (*12*). This dynamic model simulates multienzyme digestion, absorption of digested products, and physiological pH values in different parts of the gastrointestinal tract combined with physiological transit times. The model has previously been employed in experiments with modified transit times. Minekus et al. (*13*) used it to study the efficacy of phytase in a porcine stomach, partly by altering the gastric emptying rate. In the present investigation we chose to study three different meals consisting of lactic fermented vegetables with white or whole meal bread and of sourdough-fermented rye bread with a low content of phytate.

Our objective was to study if the transit time in the stomach and small intestine would affect the level of soluble iron in a meal using the mentioned in vitro model of the gastrointestinal

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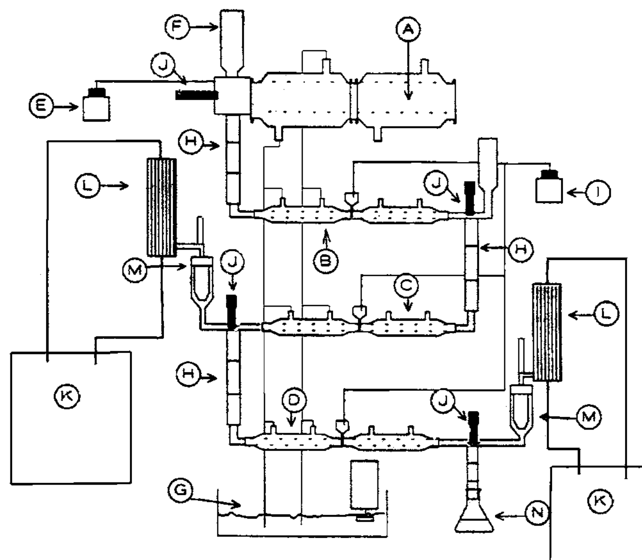

Figure 1. Schematic picture of the dynamic in vitro gastrointestinal model used in the study: (A) stomach; (B) duodenum; (C) jejunum; (D) ileum; (E) secretion pumps for stomach; (F) cardiac orifice; (G) water bath (37 ± 1 °C); (H) peristaltic valves; (I) secretion pumps for intestine; (J) pH electrodes; (K) dialysis bag; (L) dialysis filter; (M) prefilter; (N) ileal delivery.

tract. Since dialyzed, or soluble, iron may not accurately predict iron availability, we also applied the dialysates obtained from the gastrointestinal model on Caco-2 cells, which are frequently used as a model of the small intestinal epithelium (*14*−*16*). We could thus also observe the in vitro uptake of iron from the meals.

MATERIALS AND METHODS

Test Meals. Three different meals were used. Meal I consisted of 50 g of lactic fermented vegetables, 44 g of bread made from wheat flour, and 1.7 mg of Fe as $FeSO_4$, while meal II consisted of 50 g of lactic fermented vegetables, 44 g of bread made from whole meal flour, and 1.1 mg of Fe as $FeSO_4$. The lactic fermented vegetables consisted of a mixture of grated raw vegetables (40% carrots, 25% turnips, 15% white cabbage, and 20% equal parts of parsnip, celery, and onion). To 500 g of vegetables, 200 mL of sodium chloride solution (1.5%) and 0.05 g of a starter culture, *Lactobacillus pentosus* (Vege-Start 10, Christian Hansen's Lab, Denmark), were added. The lactic fermentation was performed at 20 °C for 1 week. The fermented vegetables were stored at 4 °C for 3 weeks before use. Meal III consisted of 120 g of sourdough-fermented whole-meal rye bread, and no iron was added to this meal. The bread was made by initially mixing 500 g of whole-meal rye flour, 25 g of yeast, and 750 mL of water and fermenting the mixture for 48 h at 23 °C. The sourdough formed was then mixed with 40 g of yeast, 500 g of white wheat flour, 35 g of sugar, and 10 g of table salt and kneaded. After 1 h fermentation, rolls were formed from 70 g of dough and fermented for another 20 min, followed by baking at 250 °C for 15 min (*17*).

Gastrointestinal Model. *Chemicals*. Pepsin A from porcine stomach mucosa (2260 units/mg, P-7012), trypsin from bovine pancreas (7500 BAEE units/mg, T-4665), bile extract from porcine (B-8631), and pancreatin from porcine pancreas (4 x U.S.P., P-1750) were all purchased from Sigma-Aldrich (Stockholm, Sweden). Lipase from Rhizopus lipase (150000 units/mg, F-AP 15) was an appreciated donation from Amano Enzyme Inc. (Nagoya, Japan). All other chemicals used were of reagent grade and purchased from Scharlau Chemie S.A. (Barcelona, Spain).

Description of the Model. The dynamic in vitro model is a unique model of the stomach and small intestine (**Figure 1**), which has been described in detail by Minekus et al. (*12*). Briefly, the model consists of four sections of flexible silicone tubing representing the stomach, duodenum, jejunum, and ileum. They are connected by peristaltic valves, which also settle the rate of transport of the food. To imitate peristalsis, the tubes are squeezed periodically. This is achieved by pump action on the surrounding water, which also keeps a physiological temperature (37 ± 1 °C). Secretion of digestive juices and pH-adjustment in each section are simulated according to physiological data. All these functions are controlled by a computer, and the specific parameters for each experiment are defined in different protocols. These protocols were selected according to the type of meal used to simulate the in vivo physiological response as close as possible. For simulation of the absorption of nutrients, hollow fiber membrane devices (Hospal hemodialyzer HG-400, Gambro, Renal Products, Lund, Sweden) were used with a molecular weight cut off of approximately 3000−5000 Da. Products of digestion, water, and other small molecules were collected from the jejunal and ileal compartments by pumping dialysis liquid through the semipermeable hollow fiber membrane units.

Solutions Used in the Model. Stomach part: HCl (1 mol/L), stomach electrolyte (14.75 mmol/L KCl, 53.04 mmol/L NaCl, 1.02 mmol/L $CaCl_2{\cdot}2H_2O$, 7.14 mmol/L $NaHCO_3$), pepsin (0.28 g/L stomach electrolyte), lipase (0.25 g/L stomach electrolyte), and trypsin (0.2 g/L stomach electrolyte).

Intestinal part: $NaHCO_3$ (1 mol/L), bile extract (40 g/L), pancreatin (70 g/L), and intestinal electrolyte (8.05 mmol/L KCl, 85.55 mmol/L NaCl, 2.04 mmol/L $CaCl_2{\cdot}2H_2O$).

Transit Times. To examine the effect of the transit time on the amounts of soluble iron, we varied the times for gastric and ileal delivery in a protocol suitable for the different meals. Thus, all other parameters were identical, such as pH, volumes, and meal size, and only the time spent in the stomach and small intestine changed between the experiments. The gastric and ileal delivery of food in the model are described by curves calculated from the following formula (*18*)

$$f = (1 - 2^{-(t/t1/2)\beta}) \times 100$$

where f represents the percentage of meal delivered, $t_{1/2}$ the half-time of delivery, t the time (min), and β a parameter describing the shape of the curve. The three selected protocols correspond to a fast (gastric $t_{1/2} = 30$ min, $\beta = 1$; ileal $t_{1/2} = 160$ min, $\beta = 1.6$), medium (gastric $t_{1/2} = 70$ min, $\beta = 2$; ileal $t_{1/2} = 220$ min, $\beta = 2.5$), and slow (gastric $t_{1/2} = 80$ min, $\beta = 1.5$; ileal $t_{1/2} = 335$ min, $\beta = 3.8$) transport of the food (**Figure 2**). Each protocol was repeated three times. All experiments were terminated after 360 min.

Sampling and Analyses. *Determination of Iron in Dialysate, Vegetables, and Bread*. Dialysates were collected by pumping intestinal electrolyte through the semipermeable hollow fiber membrane units connected to the jejunal and ileal compartments. Samples of the dialysate were taken every 2 h and frozen at −18 °C until analysis. Iron content was analyzed by high-performance ion chromatography (HPIC) coupled with UV−vis detection (*19*). The dialysates (0.8 mL) were pretreated with 0.1 mL of HCl (0.5 mol/L) and 0.1 mL of ascorbic acid (0.11 mol/L), mixed, and centrifuged at 9300 g for 4 min before injection of the supernatant into the HPIC.

The vegetables and the bread were freeze-dried, homogenized, and thereafter exposed for microwave digestion (Milestone microwave laboratory system Ethos Plus, Sorisole, Italy). The digested samples (0.9 mL) were pretreated with 0.1 mL of ascorbic acid (0.11 mol/L) before HPIC analysis.

Determination of Phytate in Vegetables and Bread. Sample preparation and analysis of phytate were done according to Carlsson et al. (*20*). Briefly, the freeze-dried and ground samples were extracted with 0.5 mol/L HCl for 3 h. The extracts were centrifuged (5 min, 1100 g), and the supernatants were frozen (−18 °C). After thawing and centrifugation through an ultracentrifugal filter device (Microcon YM-30, Millipore, Bedford, MA), the samples were analyzed by high-performance ion chromatography (HPIC) coupled with UV detection.

Iron Absorption by Caco-2 Cells. *Chemicals*. Dulbecco's modified Eagle medium (DMEM) with 4.5 g/L glucose and L-glutamine, nonessential amino acids (NEAA), Penicillin/Streptomycin (PEST), and trypsin-EDTA were purchased from PAA Laboratories GmbH (Linz,

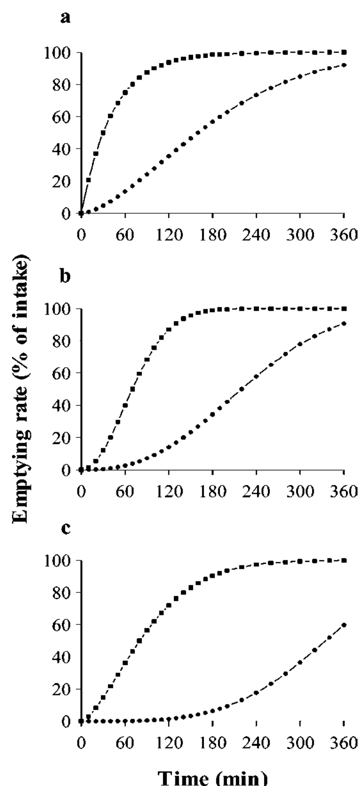

Figure 2. Illustration of the three pairs of transit times used in the experiments corresponding to (a) fast, (b) medium, and (c) slow transport of the food through the stomach and small intestine. In each figure the upper line (■) represents the gastric and the lower line (●) the ileal delivery curve.

Austria). Fetal calf serum was obtained from Biotech Line AS (Denmark), and $^{55}FeCl_3$ was obtained from NEN Life Science Products (Perkin Elmer Life Sciences Inc, Zaventem, Belgium). The LCA-cocktail, ULTIMA-FLO AP, used for scintillation counting, was purchased from Packard Bioscience B.V. (Groningen, The Netherlands). All other chemicals were purchased from Sigma-Aldrich (Stockholm, Sweden).

Cell Line and Culturing Conditions. Caco-2 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and used between passage 38 and 44. Stock cultures were maintained in 75-cm^2 flasks (TPP, Trasadingen, Switzerland) in complete medium in an atmosphere of 95% air and 5% CO_2 at 37 °C. The complete medium contained basal DMEM with 10 mL/L 100× NEAA, 10 mL/L 100× PEST, and 100 mL/L fetal calf serum. For the uptake studies cells were grown in 12-well plates (TPP, Trasadingen, Switzerland) with a seeding density of ~100 000 cells/cm^2. The medium was changed every other day and the day before using the cultures for experiments. Experiments were performed using differentiated cultures at 13−14 days post seeding.

Iron Uptake Assay. The day before the uptake assay, samples consisting of dialysates (1 pooled sample/experiment) from experiments performed in the gastrointestinal model were mixed with $^{55}FeCl_3$ to obtain 37 kBq/well and stored overnight at 4 °C on an orbital shaker (150 rpm). Prior to the uptake assay the Caco-2 cells were washed 3 times with phosphate buffer saline. The prewarmed (37 °C) samples (1 mL) were applied in duplicate or triplicate on the cells and incubated on an orbital shaker (50 rpm) for 1 h at 37 °C in air/CO_2 (95:5) atmosphere. After incubation, the sample solutions were aspirated and nonabsorbed iron was removed according to Glahn et al. (*21;22*). In brief, the cells were washed repeatedly with a stop solution (140 mmol/L NaCl, 10 mmol/L PIPES, pH 6.8, 4 °C) and 10 min with a removal solution (stop solution with 1 mmol/L bathophenanthrolinesulfonic acid and 5 mmol/L sodium dithionite, pH 6.8, 4 °C). Cells were lysed and harvested by addition of 1 mL of 0.5 mol/L NaOH; thereafter, the content of each well was homogenized by pipetting, and 0.8 mL was transferred to a scintillation vial. LCA-cocktail was added (2.5 mL), and the samples were mixed and analyzed by a Tri-Carb 1900CA liquid scintillation analyzer (Packard Instrument, Meriden, CT) to assess the amount of absorbed iron. In addition, the total amount of added radioactivity was analyzed directly by scintillation counting of 1 mL of the sample solution.

Statistical Analysis. The iron content was calculated as mean ± standard error (SE). The mean dialyzable iron for the different transit times, and the iron absorption by the Caco-2 cells was analyzed by ANOVA, after Cochran's test for homogeneity of variance. Significant differences between these groups were determined by Student-Newman-Keuls test. A P-value < 0.05 was considered significant.

RESULTS AND DISCUSSION

In Vitro Digestion. The amount of iron and phytate and the mean (± SE) amount of dialyzable iron from the different test meals are shown in **Table 1**. The level of iron present in the samples originated from two sources, the meal and the endogenous secretions (294 ± 6 μg). A significantly ($P = 0.001$) higher amount of dialyzed iron was found for meal I with the slow transit time compared with the other two transit times. For meal III, a significantly ($P = 0.01$) lower amount of dialyzed iron was found with the fast transit time compared with the other two transit times.

For meal II, with lactic fermented vegetables and whole meal bread, the lack of significant differences between the three transit times can be explained by the level of inhibitors, i.e., phytate, present in the meal (172.2 μmol of phytate or 32 mg of phytate-P). Phytate forms insoluble complexes with iron, and these are not dialyzable. Several human studies have shown that phytate is a powerful inhibitor of iron absorption, and the inhibiting effect is strong even with small amounts of phytate; more than 10 mg of phytate-P (54 μmol of phytate) per meal is considered inhibitory (*23*, *24*). Brune et al. (*17*) compared human iron absorption from bread fermented in different ways and demonstrated that effective fermentation of whole meal bread resulting in phytate reduction markedly improved iron absorption.

In choosing the third test meal, the intention was to achieve a relatively high natural iron content with a high bioavailability. Previous studies (*17*, *25*) have shown that the phytate content of rye bread can be reduced almost completely by sourdough fermentation. The sourdough rolls used contributed only with 0.8 μmol of phytate (0.15 mg of phytate-P) to the meal, which is below the level that significantly inhibit iron absorption from a meal (*24*). Hence, the possible inhibition exerted by phytate was negligible, and we expected a relatively high availability of the iron in the meal. The results indeed showed a much higher level of soluble iron from meal III, with low phytate, than from meal II, with high phytate content. The highest level of dialyzable iron was found in meal I, consisting of lactic fermented vegetables and white bread. The promoting effect of the lactic fermented vegetables in this meal was previously observed both in experiments done in an in vitro digestion/Caco-2 cell model (*26*) and in a human study (*27*).

The protocol for the medium transit time is the one most suitable for this type of meal. The selection of the three transit times was based upon existing protocols for the in vitro model, all developed from human trials. The selection criteria were to have one suitable transit time for the test meal (medium) and two extreme transit times. These additional transit times were adopted from protocols developed for a water meal, which has a very fast transit time, and a pasta meal, which has a relatively slow transit time through the gastrointestinal tract. The reason

Table 1. Amount or Iron and Phytate from the Different Test Meals, and the Amount of Dialyzable Iron Resulting from Three Different Transit Times in the in Vitro Gastrointestinal Model[a]

meal	total amount of Fe in meal (μg)	total amount of phytate in meal (μmol)	amount of dialyzed Fe after digestion in GI model (μg of Fe and % of total) fast	medium	slow
I. lactic fermented vegetables and white bread	1824 ± 1	0.1 ± 0.0	235 ± 11 μg[a] 12.9%	295 ± 15 μg[a] 16.2%	404 ± 47μg[b] 22.1%
II. lactic fermented vegetables and whole meal bread	1709 ± 2	172.2 ± 0.7	3 ± 1 μg[a] 0.2%	13 ± 13 μg[a] 0.7%	11 ± 6 μg[a] 0.7%
III. sourdough- fermented rye bread	2326 ± 10	0.8 ± 0.0	116 ± 20 μg[a] 5.0%	171 ± 23 μg[b] 7.4%	181 ± 18 μg[b] 7.8%

[a] Values in the same row not showing same superscript were significantly different ($P < 0.05$). Values are mean ± SE, $n = 3$.

Table 2. Uptake of Iron from in Vitro Digestion Dialysates in Caco-2 Cells, Expressed as μg of Fe Absorbed/h[a]

meal	iron uptake by Caco-2 cells from in vitro digestion dialysates (μg of Fe absorbed/h) fast	medium	slow
I. lactic fermented vegetables and white bread	6.81 ± 0.41[a]	8.43 ± 0.50[b]	9.14 ± 0.27[b]
II. lactic fermented vegetables and whole meal bread		0.17 ± 0.02[a]	
III. sourdough-fermented rye bread	0.88 ± 0.08[a]	1.01 ± 0.03[b]	0.82 ± 0.02[a]

[a] Values in the same row not showing same superscript were significantly different ($p < 0.05$). Values are mean ± SE, $n = 6$.

for the small differences in iron uptake between the medium and slow transit time for meal III could at least in part be that the two experiments had very similar gastric delivery curves (gastric $t_{1/2}$ was 70 and 80 min, respectively). The period of time spent in the acidic environment of the stomach has previously been suggested to be important for iron solubilization (*4*, *6*).

One advantage of the gastrointestinal model is that the transit time can be programmed. Furthermore, the effect of changing one single parameter can be investigated, experiments can be repeated several times under identical conditions, and there is no influence of the physiological status of the subject. However, this model only gives information about the amount of dialyzable iron, which is potentially available for absorption, and not the actual absorption. To improve the assessment of iron availability, dialysates from the gastrointestinal model were radioactively labeled and applied onto Caco-2 cells to study the iron uptake. By combining the two methods we also take into account the possible effect of pancreatic and biliary ligands formed during digestion on iron absorption. Han et al. (*28*) suggested that these compounds might enhance the uptake of iron at the brush border.

Fe Absorption by Caco-2 Cells. The results from the combined in vitro digestion and Caco-2 cell experiments are shown in **Table 2**. The amount of available and absorbed iron increased with prolonged transit time for meal I ($P = 0.002$). For meal III, an enhanced iron uptake could be observed only for the medium transit time ($P = 0.05$) and not for the slow transit time. Neither did the amount of dialyzable iron found increase with the slow transit time for this meal, which suggests that there may be some factor in the meal that prevents further increase of iron dialyzability and uptake. The lowest iron uptake in the Caco-2 cells was observed for meal II, containing high levels of phytate. For practical reasons only the dialysate from the medium transit time of meal II could be used in the Caco-2 cell experiments.

In our assay the dialysates were applied on the Caco-2 cells for the same length of time, 1 h, independent of transit time. However, in vivo the iron leaving the stomach would be exposed to intestinal epithelial cells for different time periods depending on transit time. Thus, with a slow transit time, the iron would have a longer time of exposure to the epithelial cells and would probably also result in a higher absorption than with a fast transit time. This would further increase the differences observed between the results displayed in **Table 2**.

The in vitro studies can only be used to predict the relative absorption from different meals. The iron absorption of all three meals has previously been studied in humans using radionuclide technique. The mean individual iron absorption from meal I (white bread and lactic fermented vegetables) was 23.6 ± 2.0% ($n = 8$) (*27*), for meal II (wholemeal bread and lactic fermented vegetables) it was 10.4 ± 2.4% ($n = 8$) (*27*), and for meal III (sourdough-fermented rye bread) it was 23.6 ± 5.6% ($n = 10$) (*17*). Thus, the meals could be ranked according to human iron absorption as follows: I = III > II. The order found in the in vitro gastrointestinal model and in the in vitro model combined with Caco-2 cells was I > III > II, which is similar to the in vivo order. Even if both meal I and III had a low phytate content and similar iron in our study, there was still a large difference in the level of dialyzed and absorbed iron between the two meals. This suggests the presence of some compound(s) from meal I enhancing both the dialyzability of iron and sequential uptake in Caco-2 cells, e.g., organic acids.

A drawback of the combination of the two in vitro methods is the need to add radioactive iron after the digestion process. Nonetheless, we tried to minimize this source of error by adding the ^{55}Fe the day before the experiments and placing the samples on an orbital shaker to allow complete isotope exchange between the ^{55}Fe and the iron in the dialysate. The fact that the Caco-2 cells lack a mucus layer may also be a source of error, since a study by Powell et al. (*29*) suggests that the intestinal mucus is a primary regulator of the absorption of metal ions.

The trends found in our study are similar to those found by Smeets-Peeters et al. (*30*) when studying the effect of transit time on calcium. They used a similar experimental design but adjusted the in vitro gastrointestinal model to mimic the gastrointestinal tract of the dog instead. The results showed a high resemblance with the in vivo situation in dogs, and the faster the transit time, the less calcium was found in the dialysis fluid. This trend was also observed for calcium absorption in

humans (*31*). The authors of the human study hypothesized that slower gastric emptying would prolong the time of supply of calcium to the intestine, consequently increasing the contact time between the calcium ions and the intestinal mucosa. Since the observations for calcium from the human study (*31*) are in accordance with those in the in vitro gastrointestinal model (*30*), it is likely that our in vitro results on iron may also be valid for a similar situation in humans.

To our knowledge only three studies have previously investigated the effect of prolonged transit time on iron availability. Schade et al. (*32*) measured iron absorption in rats treated with drugs that decreased the intestinal motility. They found that iron absorption could be enhanced by reducing the intestinal motility and increasing the exposure time of luminal iron to absorptive cells. Another study in rats also altered the transit time through the small intestine with drugs, but in this study no effect on iron absorption was observed (*33*). The use of rats as a model to predict human iron absorption has, however, been criticized (*34*, *35*). The third study on transit times was made in humans. It showed no correlation between half-time of gastric emptying and iron absorption among subjects eating a conventional meal, while homogenization of an identical meal prolonged the gastric emptying time with 31% and increased the nonheme iron absorption with 22% (*36*). However, it was not possible to determine whether the alteration in iron absorption was due to changes in gastric emptying or in the physical form of the meal. It is thus difficult to compare the results from this human study with the results from our in vitro study.

A possible method to study the effect of altered transit times on human iron absorption is to use intestinal intubation. However, this method can delay gastric emptying and shorten small intestinal transit time in human volunteers. Instead, Holgate and Read (*37*) used patients with terminal ileostomies to investigate the relationship between transit time and absorption of a solid meal in the small intestine. By using three different agents they managed to reduce the transit time for the meal, which resulted in significant reductions in the absorption of fat, carbohydrate, protein, water, and electrolytes for two of the agents. The method of using patients with terminal ileostomies might be a possibility to confirm our results in humans.

There have been reports on several natural ways to alter the gastric emptying rate or the transit time through the gut. For example, sugars (glucose and galactose) have been found to prolong the mean transit time (*31*), whereas organic acids, such as tartaric and citric acid, slowed gastric emptying in dogs (*38*) and in rats (*39*). Both acetic acid (*10*) and sodium propionate (*11*) added to bread slowed gastric emptying in humans. Hence, it should be possible to create a meal that would prolong the transit time, for example, with fermented foods containing organic acids. This would be likely to result in enhanced iron absorption and thereby an improved iron status. Perhaps other health benefits could also be expected, as certain fermented foods have a low glycemic index.

To conclude, our results show that the dialyzable iron from test meal, as measured in an in vitro gastrointestinal model, was improved with increasing transit time through the stomach and the small intestine. The combined in vitro digestion and iron absorption by Caco-2 cells confirmed an enhanced iron uptake. Although these in vitro models have limitations as compared to in vivo experiments, the results can be of significance for understanding factors influencing iron absorption and for development of food products with improved iron availability.

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